REMARKS

Claims 24-29 were examined in the Office Action dated January 15, 2002. Claims 26-29 were allowed and Claims 24-25 were rejected under 35 U.S.C. § 102(b). The rejection is traversed for reasons discussed below.

Formal Matters:

Applicants are submitting a Sequence Listing as requested and have amended the specification to insert the sequence identifiers.

Applicants submitted an Information Disclosure Statement February 12, 2001 and a Supplemental Information Disclosure Statement August 1, 2001 and have not yet received the initialed 1449 forms from the Examiner. According, copies of these 1449s accompany this response. Applicants request that the Examiner initial and return the forms, indicating that the cited references have been considered.

Overview of the Above Amendments and New Claims:

Applicant has amended the specification to comply with 37 C.F.R. § 1.821(a)(1) and (a)(2). Additionally, the sequence occurring at page 31, line 24 has been converted to upper case letters.

Applicant has canceled dependent claim 25 without prejudice and amended independent claim 24 to recite the invention with greater particularity. Specifically, claim 24 now recites that the second recombinant AAV virion comprises a coding sequence encoding a retinoid-X-receptor (RXR), the subject matter of now-canceled claim 25. Applicant has also added new claims 30-37. Independent claim 32 recites the use of three separate AAV vector constructs and independent claim 35 pertains to transduction of a mammalian cell comprising retinoid-X-receptor (RXR).

Support for the above amendments and new claims may be found throughout the specification, for example, at page 17 and page 21.

Rejection Under 35 U.S.C. § 102(b):

Claim 24 stands rejected as anticipated by Evans et al., WO97/38117 ("Evans", "the Evans PCT"). The Office contends that the Evans PCT is anticipatory by disclosing a "method of inducing expression of an exogenous gene in a mammalian subject containing (i) a DNA construct comprising an exogenous gene under the control of an EcRE, (ii) DNA encoding an EcR under the control of an inducible promoter and, optionally in the further presence of a receptor capable of acting as a silent partner therefore, binds to said EcRE, and (iii) a ligand for said EcR..." Further, the Examiner indicates that "recombinant AAV virions and AAV vectors appropriate for gene therapy applications" are disclosed in U.S. Patent No. 5,252,479 ("Srivastava", "the Srivastava patent"), which Evans incorporates by reference. Office Action, p. 3. Applicant respectfully submits that the Examiner's rejection is improper.

In order to be anticipatory, the single reference cited by the Office must disclose each and every element of the claims. *Hybritech v. Monoclonal Antibodies*, 231 USPQ 81 (Fed. Cir. 1986). Further, there must be no difference between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the field of the invention. *Scripps Clinic & Research Foundation v. Genentech*, Inc., 18 USPQ2d 1896 (Fed. Cir. 1991). In the present case, Evans fails to disclose all of the elements of the claimed invention and thus cannot be considered anticipatory.

Applicant's invention requires the use of *at least two different AAV vectors* to deliver an ecdysone receptor (EcR) and a polynucleotide of interest linked to a transcriptional promoter region having an ecdysone-responsive element (EcRE). Evans fails to disclose the use of AAV vectors in any context, especially the use of multiple AAV vector constructs in combination.

Furthermore, the Srivastava patent, incorporated by reference in Evans, fails to cure this defect. The Examiner indicates that Srivastava (incorporated by reference in the Evans PCT) "discloses recombinant AAV virions and AAV vectors appropriate for gene therapy applications." However, Srivastava discloses

nothing more than the use of single AAV vectors, and it provides no teachings whatsoever relating to Applicant's claimed invention (the use of multiple AAV vector constructs in gene regulation). Thus, the Evans PCT fails to supply all the present claim limitations, even after incorporating the Srivastava patent in its entirety; therefore, it fails as an anticipatory reference. Accordingly, Applicant requests that the Examiner withdraw her rejection under 35 U.S.C. § 102(b).

Applicant's Claimed Invention is Nonobvious:

Although the Office has not made a rejection under 35 U.S.C. § 103, Applicant addresses nonobviousness here in order to expedite allowance and remove any further potential issues.

The law is clear that three basic criteria must be met to establish a *prima facie* case of obviousness: (MPEP §2143):

First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure (*In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1440 (Fed. Cir. 1991).

Failure to establish **any one** of these three requirements precludes a finding of a *prima facie* case and, without more, entitles applicant to allowance of the claims at issue.

As discussed in detail above, the combination of Evans et al. and Srivastava simply fails to teach all of the limitations of the claimed invention. Thus, the claims cannot be properly rejected as obvious over these references.

However, even if Evans and Srivastava did collectively contain all of the elements of the claimed invention, there would be no expectation that this combination would successfully achieve the invention. That is, based on these

references, there would be no expectation that (1) the multiple AAV vectors required would transduce the same cells, and (2) even if the cells were multiply transduced with virions containing each of the required AAV vector constructs, that the EcR and/or RXR sequences would be transcribed and translated at levels sufficient to block the transcription of the polynucleotide of interest in the absence of ecdysone or an ecdysone analog.

In sum, the cited art fails to provide a sufficient basis for either a rejection under 35 U.S.C. § 102(b) or 35 U.S.C. § 103 as it fails to disclose all of the elements of the Applicant's claimed invention. Moreover, the skilled artisan would not reasonably expect that transducing a mammalian cell with multiple different AAV vectors would provide a fully functioning inducible gene expression system. Nothing in the literature suggests this possibility. Therefore, applicant's claimed invention must be viewed as novel and non-obvious in light of Evans and Srivastava.

CONCLUSION

Applicant respectfully submits that the present claims are patentable. If the Examiner notes any further matters which she believes may be resolved by a telephone interview, she is encouraged to contact M. Christina Thomson by telephone at (510) 748-7208 or by fax at (510) 748-7368.

Respectfully submitted,

Date: 4/11/02

By: _____
Roberta L. Robins
Registration No. 33,208

ROBINS & PASTERNAK LLP
545 Middlefield Road, Suite 180
Menlo Park, CA 94025
Telephone: (650) 325-7812
Fax: (650) 325-7823

Version with markings to show changes made

Claim 24 has been amended as follows:

24. (Amended) A method of inducing gene expression in a mammalian cell, said method comprising:

(a) transducing the mammalian cell with (i) a first recombinant adeno-associated virus (AAV) virion comprising an AAV vector that comprises a transcriptional promoter region operably linked to a polynucleotide of interest, wherein the transcriptional promoter region comprises at least one ecdysone-responsive element (EcRE), and a promoter capable of directing the *in vivo* transcription of said polynucleotide of interest in a mammalian cell, located downstream of the at least one EcRE; and (ii) a second recombinant AAV virion comprising an AAV vector that comprises a coding sequence encoding an ecdysone receptor (EcR) and further comprises a coding sequence encoding a retinoid-X-receptor (RXR), wherein said EcR and RXR coding sequences are operably linked to control elements capable of directing the *in vivo* transcription [of said EcR coding sequence] thereof in the mammalian cell; and

(b) providing ecdysone, or an analog thereof capable of binding the EcR, to said mammalian cell, in an amount sufficient to induce expression of the polynucleotide of interest.

Claim 25 has been canceled.

Claims 30-37 have been added:

--30. (New) The method of claim 24, wherein the transcriptional promoter region of the AAV vector of the first recombinant AAV virion further comprises at least one enhancer sequence.

31. (New) The method of claim 30, wherein the enhancer sequence is an SP1 enhancer sequence.

32. (New) A method of inducing gene expression in a mammalian cell, said method comprising:

(a) transducing the mammalian cell with (i) a first recombinant adeno-associated virus (AAV) virion comprising an AAV vector that comprises a transcriptional promoter region operably linked to a polynucleotide of interest, wherein the transcriptional promoter region comprises at least one ecdysone-responsive element (EcRE), and a promoter capable of directing the *in vivo* transcription of said polynucleotide of interest in a mammalian cell, located downstream of the at least one EcRE; (ii) a second recombinant AAV virion comprising an AAV vector that comprises a coding sequence encoding an ecdysone receptor (EcR) operably linked to control elements capable of directing the *in vivo* transcription thereof in the mammalian cell; and (iii) a third recombinant AAV virion comprising an AAV vector that comprises a coding sequence encoding a retinoid-X-receptor (RXR) operably linked to control elements capable of directing the *in vivo* transcription thereof in the mammalian cell; and

(b) providing ecdysone, or an analog thereof capable of binding the EcR, to said mammalian cell, in an amount sufficient to induce expression of the polynucleotide of interest.

33. (New) The method of claim 32, wherein the transcriptional promoter region of the AAV vector of the first recombinant AAV virion further comprises at least one enhancer sequence.

34. (New) The method of claim 33, wherein the enhancer sequence is an SP1 enhancer sequence.

35. (New) A method of inducing gene expression in a mammalian cell, said method comprising:

(a) transducing a mammalian cell comprising a retinoid-X-receptor (RXR) with (i) a first recombinant adeno-associated virus (AAV) virion comprising an AAV vector that comprises a transcriptional promoter region operably linked to a polynucleotide of interest, wherein the transcriptional promoter region comprises at least one ecdysone-responsive element (EcRE), and a promoter capable of directing the *in vivo* transcription of said polynucleotide of interest in a mammalian cell, located downstream of the at least one EcRE and (ii) a second recombinant AAV virion comprising an AAV vector that comprises a coding sequence encoding an ecdysone receptor (EcR) operably linked to control elements capable of directing the *in vivo* transcription thereof in the mammalian cell; and

(b) providing ecdysone, or an analog thereof capable of binding the EcR, to said mammalian cell, in an amount sufficient to induce expression of the polynucleotide of interest.

36. (New) The method of claim 35, wherein the transcriptional promoter region of the AAV vector of the first recombinant AAV virion further comprises at least one enhancer sequence.

37. (New) The method of claim 36, wherein the enhancer sequence is an SP1 enhancer sequence.--

The specification has been amended as follows:

The paragraph beginning on page 30, line 7 has been amended as follows:

p4.1c: A synthetic DNA encoding the restriction enzyme sites NotI-MluI-Ecl136II-SstII-SfuI-SmaI-SfuI-ClaI-BglII-SnaBI-BstEII-PmlI-RsrII-NotI and having the sequence
(CGGCCGCACGCGTGAGCTCCGCGGTTCGAATCCCGGGATTCGAACAT
CGATAAAAGATCTACGTAGGTAACCACGTGCGGACCGAGCGGCCGC)
(SEQ ID NO:1) was cloned into the blunted KasI and EarI(partial) sites of pUC119 (the vector fragment is 2757bp in length). A 653bp SpeI(blunted)-SacII(blunted) fragment encoding the CMV immediate early (IE) promoter, and a 488bp, SmaI-DraIII fragment containing the human growth hormone polyadenylation site, were cloned into the Ecl136II and SnaBI sites of the afore-mentioned plasmid, respectively. A chimeric intron composed of the splice donor from the first intron of CMV IE gene and the splice acceptor from the second intron of the human ß- globin gene was then installed into the SmaI site of the plasmid in two steps. A DNA fragment encoding the CMV IE gene first intron splice donor was produced by PCR using isolated CMV DNA (strain ad169) as template and the following primers, GGCCGGGAACGGTGCATT (SEQ ID NO:2), and GGGCAAGGGGGTGGGCCTATA (SEQ ID NO:3). This 87 bp fragment was ligated into the SmaI site of the plasmid intermediate. The resulting plasmid was cleaved with BstXI and SmaI, blunted with T4 DNA polymerase, and a 398bp DraI-EcoRI(blunt) fragment encoding the human ß-globin second intron splice acceptor was ligated into the plasmid. The construction of p4.1c was completed by ligation of a polylinker encoding the restriction sites ClaI-EcoRI-SmaI-BamHI-XbaI-SalI-PstI-HinDIII-XhoI-Eco47III-XhoI-BglII between the ClaI and BglII sites of the last intermediate plasmid. The sequence of this synthetic DNA was
ATCGATTGAATTCCCCGGGGATCCTCTAGAGTCGACCTGCAGAAGCTT
GCTCTCGAGCAGCGCTGCTCGAGAGATCT (SEQ ID NO:4).

The paragraph beginning on page 31, line 1 has been amended as follows:

p4.1c mEPO: p4.1c was digested with SmaI and a 2812bp SmaI(partial)-NcoI(blunted) fragment encoding all of the exons of the mouse erythropoietin gene was inserted. The Kozak sequence around the initiator methionine was changed to the optimally translated sequence, CCACCATG (SEQ ID NO:5), using oligonucleotide directed mutagenesis. The sequence of the mutagenic oligonucleotide was AGCTAGGCGCCACCATGGGGGTGC (SEQ ID NO:6).

Please replace the paragraph beginning on page 31, line 8 with the following rewritten paragraph:

pV4.1c mEPO: The polylinker and lacZ alpha fragment expression cassette of pUC119 was replaced by a single Sse8387I site by ligation of the following synthetic DNA fragment in the plasmid vector after digestion with AflIII and EheI, GGCGCCCCTGCAGGACATGT (SEQ ID NO:7). The resulting plasmid was cut with Sse8387I and the 4772bp Sse8387I fragment from pW1909adhlacZ that contains the ITR-bounded lacZ expression cassette was ligated to it. The resulting plasmid was called intermediate1. Next, p4.1c mEPO was digested with NotI and the 4582bp fragment encoding the mEPO expression cassette was isolated. One copy of a synthetic DNA fragment that encodes the D region of the AAV ITR was ligated to each end. The sequence of this synthetic fragment was GCGGCCGCAGGAACCCCTAGTGATGGAGTTGG (SEQ ID NO:8). The product of this reaction was ligated into the 2831bp, plasmid vector encoding MscI fragment of intermediate1(above) to form pV4.1c mEPO.

Please replace the paragraph beginning on page 31, line 21 with the following rewritten paragraph:

p4.1c hEPO: p4.1c was cleaved with SmaI and the 718bp, PpuMI-NcoI fragment of the human Epo cDNA (blunted) was ligated into this site. The translational initiation sequence was then modified by oligonucleotide -directed mutagenesis using the following mutagenic oligo: [catcgattgaattccaccatgggggt]

CATCGATTGAATTCCACCATGGGGGT (SEQ ID NO:9). The resulting construct was cleaved with Pml I and the 1765bp, EcoRV-HincII fragment of the LacZ gene was ligated into it.